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Application News

No. O73

Total Organic Carbon Analysis

Analysis of Microalgae Biomass by TOC and TG

Microalgae have various attractive features, as they can be used to produce oil without competing with food production. In comparison with other biofuels, productivity per unit time and area is higher and there are many choices of cultivation site. Microalgae display different growth behaviors depending on cultivation conditions, and it is considered necessary to understand the changes in their organic carbon and inorganic carbon contents. A total organic carbon (TOC) analyzer can be used for the analysis of these carbon components. Moreover, direct analysis of microalgae in the wet state is possible if a solid sample combustion unit is used, and the carbon content in the dried microalgae can also be evaluated easily by using the combustion unit in combination with thermogravimetry (TG) analysis. This article introduces analysis results of three species of microalgae.

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Samples

Three species of microalgae were used in this experiment, the haptophyte coccolithophore *Emiliania huxleyi* NIES-837 (Alga A), the haptophyte isochrysis *Tisochrysis lutea* (Alga B), and the green chlorella *Chlorella vulgaris* (Alga C). The specimens were provided by Prof. Yoshihiro Shiraiwa of the University of Tsukuba.

Algae A to C were cultured for approximately 10 days under set conditions, and culture solutions containing suspended microalgae were prepared (Fig. 1). These microalgae suspensions were subdivided into bottles, and samples were obtained by collecting only pellet-like cultured cells, which were the sedimentation component, after repeatedly performing centrifugal separation and washing the culture medium (Fig. 2).

Analytical Method

A total carbon (TC) analysis of the pellet-like microalgae samples was carried out by using a TOC solid sample analysis system comprising a TOC-L and an SSM-5000A solid sample combustion unit, which is an option of the TOC analyzer (Table 1). The moisture content was evaluated from the results of TG analysis with a DTG-60 simultaneous thermogravimetric/differential thermal analyzer (Table 2).

Table 1 Conditions of TOC-L + SSM-5000A Analysis

Instrument	:	TOC solid sample analysis system (TOC-L_{CPH} + SSM-5000A solid sample combustion unit)
Cell length	:	Short cell
Carrier gas	:	Oxygen, 500 mL/min
TC oxidation method	:	Combustion catalytic oxidation (combustion temperature: 900 °C)
Measurement item	:	TC (total carbon)
Calibration curve	:	One-point calibration curve prepared using 40 % carbon glucose powder

Table 2 Conditions of DTG-60 Analysis

Instrument	:	DTG-60
Sample pan	:	Platinum
Ambient gas	:	Air
Heating rate	:	20 °C/min
Holding temp.	:	800 °C

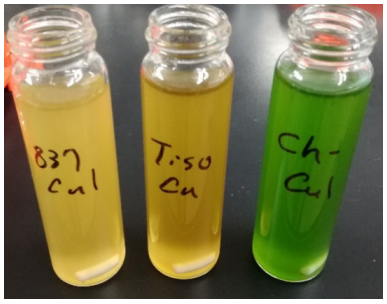

Fig. 1 Culture Solutions Containing Suspended Microalgae (from Left, A, B, and C)

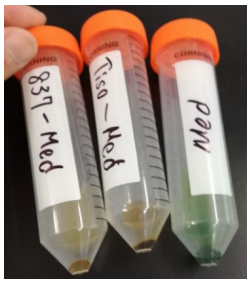

Fig. 2 Pellet Cultured Cells

Analysis Results

(1) TC Analysis of Pellet-like Microalgae Samples

In order to evaluate the appropriateness of the cultivated cell analysis method using the TOC solid sample analysis system, the linearity of the pellet weight and TC was evaluated. Pellet cells of Algae A to C were divided into several weights in the range of approximately 20 mg to 100 mg and weighed, and a correlation graph of the pellet weight and TC was prepared.

As a result, the relationship of TC to pellet weight showed satisfactory linearity, as shown in Fig. 3. Based on this result, it was found that the carbon content in pellet cells recovered by centrifugal separation is uniform, and deviations in the carbon content due to sampling do not occur. This correlation result shows that correct TC values can be calculated when sampling the different weight of pellet.

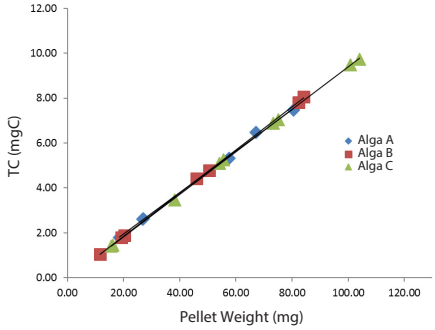

Fig. 3 Correlation Diagram of Pellet Weight and TC

(2) Analysis of Moisture Content of Pellet-like Microalgae Samples

The DTG-60 simultaneous thermogravimetry/differential thermal analyzer is an instrument that analyzes the thermal characteristics of sample materials by the weight changes due to dewatering, sublimation, evaporation, decomposition, combustion, and other reactions which occur due to heating.

Pellets of Algae A to C recovered by centrifugal separation were placed and weighed in the sampling pan of the DTG-60 without pretreatment, heated from room temperature to 800 °C, and measured, as organic matter is completely combusted at 800 °C. Fig. 4 shows the data for Alga A measured with the DTG-60.

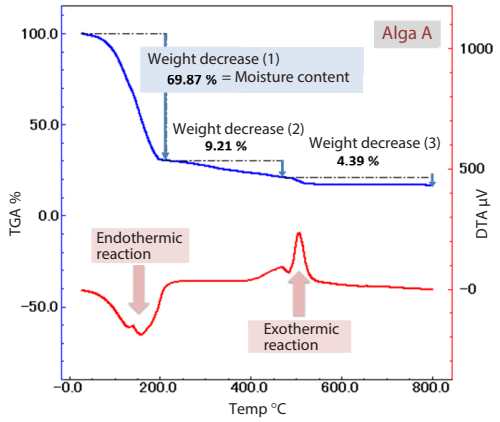

Fig. 4 Data for Alga A Measured with DTG-60.

The abscissa of the DTG-60 graph shows temperature change, the left ordinate shows weight change (thermogravimetric analysis: TGA %), and the right ordinate shows the change in heat quantity (differential thermal analysis: DTA µV). A remarkable weight decrease occurred below 200 °C. Subsequently, slight weight decreases were also observed in the medium temperature region and the high temperature region. Because the microalgae pellets contain water that cannot completely separated by centrifugal separation, a weight decrease due to the loss of this moisture content occurred in the low temperature region. (This decrease is equivalent to "Weight decrease (1)" in the DTG-60 measurement data.) This weight decrease is considered to represent the content of moisture contained in the pellets. The slight weight decreases in the mid- and high-temperature range may reflect volatilization, degradation, and combustion caused by the structures and components retained in the dry cells themselves of the respective algae.

Table 3 summarizes the moisture contents from the DTG-60 data obtained by measurement of pellets of Algae A to C.

Table 3 Moisture Contents of Algae A to C

Alga	Moisture Contents (%)
A	69.87
B	71.47
C	71.60

Conclusion

Calculation of TC Contained in Dried Cultured Cells

It was possible to analyze the TC in pellets of microalgae cells by using the TOC solid sample analysis system and obtain the moisture content in the pellets from the results of TG analysis. It was also possible to calculate the TC of dry cultured cells for completely dried pellets from the TC and moisture content of each type of pellet. The correlation graph of the dry cultured cell weight and TC shown in Fig. 5 was prepared by converting the wet pellet weight by the moisture content.

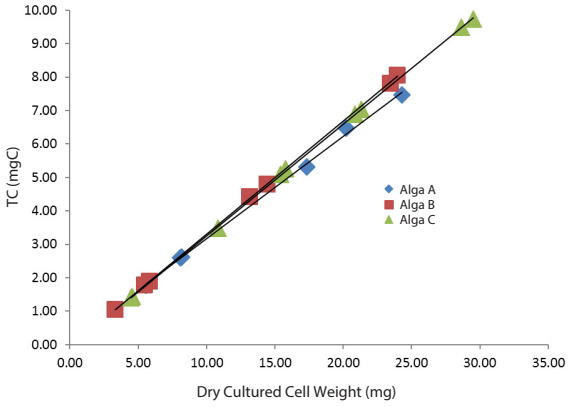

Fig. 5 Correlation Graph of Dry Cultured Cell Weight and TC

The dry weight method has historically been used as a technique for monitoring the culture condition of microalgae. With the dry weight method, the growth fraction of microalgae is evaluated with the weight of dry cells obtained by drying a solution containing suspended microalgae. However, this method has issues, in that the drying treatment is time-consuming and measurement accuracy is low.

By applying the new method introduced here using the TOC solid sample analysis system and TG analyzer, it is possible to recover pellets by centrifugal separation of a solution containing suspended microalgae, obtain information on TC by direct measurement of those pellets, and then simply calculate the TC of the dry cultured cells from the moisture content obtained by the TG analyzer. This is a very effective method in this study because of the quick and simple procedure in comparison with the dry weight method, in addition, it also enables the selective evaluation of growth fraction of the carbon content.

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